UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: July 9, 2020

SUBJECT: Comments on the Exponent Whitepaper Regarding Pharmacodynamic Parameters of Human and Rat Acetylcholinesterase Inhibition by Direct-Acting Organophosphorus (OP) Insecticides or Active Metabolites

PC Code: See table below
Decision No.: 564279
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0058058
MRID No.: 50773505

DP Barcode: D458415
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: See table below
40 CFR: NA

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CHEMICAL	PC CODE	CAS NUMBER
Bensulide	009801	741-58-2
Chlorethoxyfos	129006	54593-83-8
DDVP	084001	62-73-7
Dicrotophos	035201	141-66-2
Dimethoate	035001	60-51-5
Ethoprop	041101	13194-48-4
Fenamiphos	100601	2224-92-6
Malathion	057701	121-75-5
Methamidophos	101201	10265-92-6
Naled	034401	300-76-5
Omethoate	035002	1113-02-6
Parathion	057501	56-38-2
Phorate	057201	298-02-2
Phosmet	059201	732-11-6
Phostebupirim	129086	96182-53-5
Terbufos	105001	13071-79-9
Tribufos	074801	78-48-8

Background:

Studies to measure the acetylcholinesterase (AChE) inhibition kinetics were conducted and submitted to the Agency (MRID 50773501-50773503) by a consortium of three agrochemical companies (AMVAC, FMC, and Gowan) for several organophosphate (OP) pesticides with the goal to replace default pharmacodynamic (PD) interspecies and intraspecies factors with data derived extrapolation factors (DDEFs). These studies attempt to quantify potential differences (if any) in PD parameters for AChE inhibition between rats and humans and across the human population. To supplement these studies, Exponent has submitted an additional document (MRID 50773505) to summarize existing knowledge that may be combined with the new data to support study hypotheses. This memorandum provides a review of this supplemental document.

Comments:

Similar amino acid sequences and 3D structures

An underlying argument made throughout the supplemental document is that similar 3D structures lead to similar interactions with AChE inhibitors among the AChE enzymes in question (i.e., red blood cell (RBC) vs. brain, rat vs. human, different human lifestages) due to similar amino acid sequences. Equating structure with response, Exponent's supplemental document assumes that the structural similarities would therefore be reflected by similar PD parameters.

Exponent's supplemental document concentrates on the structure and function of the catalytic site; however, there was no discussion of additional aspects of AChE structure that may have the potential to affect its function and activity. For example, binding of compounds to the peripheral anionic site can change the interaction of AChE with its substrate and inhibit AChE activity either by changing the conformation of the gorge and catalytic site or by blocking access to the gorge (Berman et al. 1981; Johnson and Moore 2006; Radic and Taylor 1999). The peripheral anionic site is located at the rim of the gorge, while acetylcholine is hydrolyzed at the catalytic site located at the bottom of the gorge (Figure 1; Sussman et al. 1991). There is evidence (Changeux, 1966; reviewed in Johnson and Moore, 2006) that changes in the peripheral

anionic site alter the interaction of the catalytic site with the substrate, but there is sparse information on whether the binding of chemicals to the peripheral anionic site alters inhibition kinetics of the enzyme (Barak et al. 1994). Additionally, since oxon metabolites of some OPs appear to interact with both the catalytic site and the peripheral anionic site, steric interaction at these sites may lead to changes in inhibitor sensitivity of AChE dependent on oxon concentration, in contrast to the assumptions of classical inhibitory kinetics (Kardos and Sultatos 2000; Kousba et al. 2004; Rosenfeld and Sultatos 2006). The universally reported substrate inhibition of AChE is also thought to be an interaction between substrate binding at both the peripheral and catalytic sites (Colletier et al, 2006).

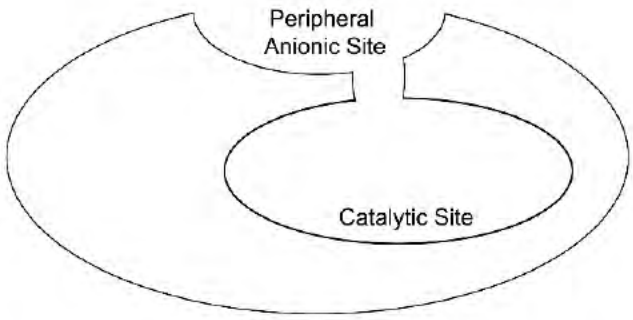

Figure 1. Illustration showing the relationship between the catalytic site at the bottom of the acetylcholinesterase gorge and the peripheral anionic site located at the mouth of that gorge. [Modified from https://proteopedia.org/wiki/index.php/Image:Sites_de_l%27AChE.jpg#filelinks, Tiphaine Jaeg]

There are a limited number of studies on other sites of AChE which affect enzyme activity and theoretically could affect the enzyme's affinity to bind to inhibitors. For example, Roca et al. (2018) used computational and experimental methods to identify novel allosteric sites on the periphery of AChE that inhibit enzyme activity. Just recently, another site has been identified which affects AChE activity (Bondžić et al. 2020). In recognition of the considerable interplay among AChE's many allosteric sites, Chandar et al. (2019) cautioned that to fully understand the interaction of AChE with an inhibitor or substrate, molecular dynamic simulations should be conducted. In sum, the acetylcholinesterase molecule is not a static molecule, but rather an extremely dynamic molecule with all parts of the molecule moving and

changing to direct the substrate and its products into and out of the gorge. Therefore, the approach of aligning 3D structures and focusing solely on the catalytic site for reaching conclusions about PD parameters may not provide a complete description of PD parameters.

PD parameters for inhibition of RBC as surrogate for brain AChE

Despite measurements of RBC AChE activity often being used as a surrogate for nervous tissue AChE activity due to limited accessibility of brain tissue, there is a sparsity of empirical evidence available to support or refute the hypothesis that RBC AChE PD parameters accurately reflect brain AChE parameters. Several journal articles were cited in Exponent's supplemental document with only a limited amount of empirical evidence (Basova and Rozengart 2009; Herkert et al. 2012). In Basova and Rozengart (2009), the authors concluded that the PD parameters of brain and RBC AChE inhibition of the same species (rat or human) using four different OP inhibitors were "very close," but there was only one observation from each tissue from each species (n=1), which precluded statistical comparisons. In Herkert et al. (2012), the inhibition of human brain and RBC AChE were found to be comparable for paraoxon inhibition but not for sarin inhibition. The human brain tissue used in those comparisons was not normal brain tissue, but rather tumor (glioblastoma) samples from only two patients; tumor cells may express abnormal forms of AChE (Karpel et al, 1994; Soreq et al, 1991). Another article (Coban et al. 2016), which was not discussed in the Exponent's supplemental document, appeared to demonstrate differences between rat brain and human RBC PD parameters; however, no formal statistical comparison was performed. The remaining papers cited in Exponent's supplemental document did not present additional empirical data comparing the PD parameters of RBC and brain AChE. If additional studies are identified in the literature or additional data are generated (preferably with tissues taken and tested from the same animals/subjects), the weight of evidence to either support or refute this hypothesis would be further strengthened.

Differences in PD parameters for human and rat RBC AChE

The studies conducted and submitted to the Agency to measure AChE inhibition kinetics following exposure to several OP pesticides (MRID 50773501-50773503) were performed to quantify potential differences in PD parameters, if any.

Differences in PD parameters across populations

The studies conducted and submitted to the Agency to measure AChE inhibition kinetics are intended to quantify potential differences in PD parameters across the human population; however, the Agency has previously raised concerns regarding the number of samples in these analyses, particularly in the stratified analyses. As noted previously, Exponent's analysis of the data focus primarily in the 3D structure and sequence homology at the AChE catalytic site. However, there is evidence suggesting that other factors such as mutations (outside of the catalytic site) and post-translational modifications may contribute to PD parameter differences across age, gender, or disease status.

With respect to changes in post-translational modifications and their potential impact on PD parameters, the only known modification of either rat or human AChE considered in Exponent's supplemental document is *N*-glycosylation and it was argued that *N*-glycosylation would not be expected to alter the catalytic properties because these modification sites are far from the catalytic site. The Exponent supplemental document also points out that mutations in *N*-glycosylation sites had no significant effect on activity or PD characteristics (Velan et al., 1993). Upon closer examination of that publication, however, it appears that Velan and coworkers demonstrated that, in some cases, the mutant:wild type IC_{50} ratio for three different inhibitors may change 2-7.5 times, meaning that preventing some patterns of post-translational modification has the potential to change the PD properties of AChE.

With respect to mutations, Exponent's supplemental document only considered mutations of the catalytic site. It has been shown in studies of insect mutants resistant to OP pesticides that although the site of the mutation conferring resistance (i.e., changing PD parameters) usually occurs in the active site gorge, mutations influencing PD parameters may also occur elsewhere on the molecule (Mutero et al., 1994; Casida and Durkin, 2013) and, therefore, need to be considered.

In order to understand variability across a population, including potential post-translational modifications and potential mutations of concern, sufficiently large and diverse data sets should be collected – to the extent possible – to address the population and/or evaluate populations deemed sensitive. In the case of the OPs, generating empirical data to measure AChE inhibition kinetics, like those submitted by the consortium of OP agrochemical companies, will likely encompass potential post-translational modifications and potential

mutations of concern that may have an impact on PD parameters. Another approach for evaluating mutations of concern would be to take RBC and brain samples possessing the common mutations and test them against the pesticide library to determine PD parameters.

Conclusions:

The supplemental document by Exponent (MRID 50773505) is a component of the data package submitted by the consortium of three agrochemical companies for the generation of DDEFs for several registered OPs (MRID 50773501-50773503). These studies were conducted to quantify potential differences, if any, in PD parameters between rats and humans and across the human population. These data have the potential to encompass potential post-translational modifications and potential mutations of concern that may have an impact on PD parameters in humans. However, the Agency has expressed concerns with the number of samples in these analyses and subsequently whether the sample set is sufficient to address the human population. The document provided an extensive summary of existing knowledge regarding AChE in rats and humans, including amino acid sequence alignments and 3D structures; however, it could benefit from discussion of additional aspects of AChE structure, beyond the catalytic site, that have the potential to affect its function and activity. Despite measurements of RBC AChE activity often being used as a surrogate for nervous tissue AChE activity, there is a sparsity of empirical evidence available to support or refute whether RBC AChE PD parameters accurately reflect brain AChE parameters. From a risk assessment perspective, this may not be essential because the individual risk assessments for OPs are based on the most sensitive compartment (i.e., RBC or brain).

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